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Quantitative prediction of histamine H1 receptor occupancy by the sedative and non-sedative antagonists in the human central nervous system based on systemic exposure and preclinical data

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ABSTRACT

Significant histamine H1 receptor occupation in the central nervous system (CNS) is associated with sedation. Here we examined the time profiles of the H1 receptor occupancy (RO) in the CNS using sedative (diphenhydramine and ketotifen) and non-sedative (bepotastine and olopatadine) antagonists at their therapeutic doses by integrating *in vitro* and animal data. A pharmacokinetic model was constructed to associate plasma concentrations and receptor binding in the brain. Dissociation and association rate constants with the H1 receptor and plasma and brain unbound fractions were determined *in vitro*. Passive and active clearances across the blood–brain barrier (BBB) were estimated based on physicochemical properties and microdialysis studies in mice and monkeys. The estimated RO values were comparable with the reported values determined at time to maximum concentration (T_{max}) of plasma by positron-emission tomography in humans. The simulation suggested that the predicted maximum ROs by bepotastine and olopatadine were greater than the reported values. Sensitivity analysis showed that active transport across BBB had a significant impact on the RO duration of the H1 antagonists examined. The present study demonstrated that modeling and simulation permits a reasonable RO estimation in the human CNS. Our findings will facilitate the development of CNS-acting drugs.

1. Introduction

Histamine H1 antagonists are widely used for relief from allergic disorders, such as rhinitis and atopic dermatitis [1]. One of their adverse effects is sedation, particularly for first-generation drugs, and the occupation of the H1 receptors in the central nervous system (CNS) is considered the underlying mechanism [2]. Positron-emission tomography (PET) studies have directly demonstrated the interaction between the H1 antagonists and the H1 receptor in the human brain, using the PET tracers, such as [^{11}C] doxepin. Following the administration of therapeutic doses of some H1 antagonists, [^{11}C]doxepin radioactivity in CNS significantly decreased. Comparing the radioactivity in patients treated with and without H1 antagonist administration reveals the receptor occupancy (RO) by the drug in CNS. Based on RO of the H1 receptor, H1

Abbreviations: AUC, area under the curve; BBB, blood–brain barrier; CNS, central nervous system; C_{blood}, blood concentration; $C_{u,brain}$, unbound concentration in brain; $C_{u,plasma}$, unbound plasma concentration; ISF, interstitial fluid; i.v., intravenous; KO mouse, knock out mouse; k_{on}, association rate constant; k_{off}, dissociation rate constant; $K_{p,uu}$, Brain-to-plasma unbound concentration ratio; LC-MS/MS, liquid chromatography tandem mass spectrometry; PBS, phosphate-buffered saline; PET, positron emission tomography; P-gp, P-glycoprotein; PK/PD, pharmacokinetics–pharmacodynamics; RAF, relative activity factor; R_B, blood-to-plasma concentration ratio; RO, receptor occupancy; T_{max}, time to maximum concentration.

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antagonists can be classified into sedative, less-sedative, and non-sedative types, which exhibit 50%–100%, 20%–50%, and 0%–20% RO, respectively [3]. Due to the RO determination at a limited number of time points in clinical studies (at the time to plasma maximum concentration (T_{max}) [4–8] or another later time point [9]), a quantitative analysis to associate the dose or plasma concentrations with RO has not been conducted for H1 antagonists. Therefore, whether H1 RO can be reasonably explained by *in vitro* data remains unknown.

RO is determined by the unbound concentration of the antagonist in CNS ($C_{u,brain}$) and its association (k_{on}) and dissociation (k_{off}) rate constants for the target receptor [10]. $C_{u,brain}$ provides a better prediction of RO in the brain than unbound blood drug concentration [11]. Whether $C_{u,brain}$ peaks at plasma T_{max} depends on the pharmacokinetic properties of the antagonist, particularly the distribution volume in the brain. Modeling and simulating RO following oral H1 antagonist administration, using pharmacokinetic parameters determined in *in vitro* or animal studies, may compensate for missing data points of clinical PET studies. We demonstrated that integration of the kinetic parameters of dopamine D2 receptor antagonists, such as quetiapine and perospirone, could account for the time profiles of the occupancy of D2 receptor and other receptors with which quetiapine and perospirone interact in the human brain [12].

Some H1 antagonists undergo active efflux at the blood–brain barrier (BBB) via P-glycoprotein (P-gp). In P-gp (*Mdr1a* or *Mdr1a/1b*) knockout (KO) mice, the brain-to-plasma concentration ratios of the H1 antagonists, bepotastine and olopatadine, were 2.9–5.3-fold higher than in wild-type mice [13,14]. Conversely, proton-coupled organic cation transport has been suggested for the uptake of diphenhydramine in human immortalized brain capillary endothelial cells [15]. Furthermore, a microdialysis study in rats demonstrated that $C_{u,brain}$ of diphenhydramine was 5.5-fold higher than the unbound plasma concentration ($C_{u,plasma}$) [16]. Therefore, a pharmacokinetic model for the CNS distribution of the H1 antagonists must take into account active influx and/or efflux across BBB.

Here, we examined the time profiles of RO by H1 antagonists in CNS based on non-clinical data (*in vitro*, animal, and *in silico* data) and blood concentration (C_{blood})-time profiles at the dose employed in the PET studies. The predicted values were compared with those observed in clinical PET studies. As model compounds, sedative H1 antagonists (diphenhydramine and ketotifen), and non-sedative H1 antagonists (bepotastine and olopatadine) were selected.

2. Materials and methods

2.1. Materials

[Pyridinyl-5-^{3}H]pyrilamine [(^{3}H)mepyramine, 20 Ci/mmol] and [^{14}C]carboxyl-inulin (2 mCi/g) were purchased from PerkinElmer (Boston, MA). Bepotastine was donated by Mitsubishi Tanabe Pharma Co. (Osaka, Japan). Pooled human serum, diphenhydramine hydrochloride, cetirizine, antipyrine, and phosphate-buffered saline (PBS) were purchased from Sigma–Aldrich Corp. (St. Louis, MO). Pooled male monkey serum was purchased from Hamri Co., Ltd. (Ibaraki, Japan). Ketotifen was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Olopatadine was from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). All other reagents were of analytic grade and obtained from conventional commercial sources.

2.2. Animals

Male *ddY* mice (8–9 weeks old) purchased from Japan SLC, Inc. (Shizuoka, Japan) were maintained under standard conditions with a reversed dark–light cycle for at least 4 days before the experiments. The animal experiments were conducted in accordance with the guidelines provided by the Institutional Animal Care Committee at the Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan. Experimental protocols and procedures for microdialysis studies in mice were in accordance with the Guidelines for Animal Care and Use of Otsuka Pharmaceutical Co, Ltd. (Tokushima, Japan).

Experimental protocols and procedures for microdialysis studies in monkeys were approved by the Institutional Animal Care and Use Committee of TransGenic, Inc. (Kumamoto, Japan) and Eisai Co., Ltd. (Ibaraki, Japan). Eight male cynomolgus monkeys (4–7 years old, 3.6–5.1 kg) were used in this study. All surgeries and procedures were performed by veterinarians at TransGenic, Inc., and the condition of animals before, during, and after surgery was closely monitored by the veterinarians. All efforts were made to minimize suffering.

2.3. Membrane preparation and binding assay

Transient expression of human H1 receptor (D28481.1) in HEK293 cells was performed using pcDNA3.1(+) (Life Technologies, Carlsbad, CA) and FuGENE HD (Roche Applied Science, Penzberg, Upper Bavaria, Germany) according to manufacturers' protocols. Membranes were prepared at 4 °C as previously described [17].

A [^{3}H]mepyramine binding assay was performed as previously described [18]. Briefly, the membranes (25–50 μg of protein) were incubated for 2–3 h at 37 °C in 0.5 mL of 50 mM Na_2/K phosphate buffer (pH 7.4) with 0.3–30 nM of [^{3}H]mepyramine to determine K_d and B_{max} values or with 3 nM of [^{3}H]mepyramine and increasing concentrations of unlabeled H1 antagonists to determine the K_i values. After incubation, the mixture was immediately poured onto glass fiber filters (24 mm, GF/C; Whatman, Inc., Clifton, NJ), which were then washed twice with ice-cold Na_2/K phosphate buffer. Non-specific binding of [^{3}H]mepyramine to membranes was determined in the presence of 10 μM cetirizine. Radioactivity was determined using a liquid scintillation counter (LS 6000SE; Beckman Instruments, Inc., Fullerton, CA).

The K_d, B_{max} and K_i values were determined using the iterative non-linear least-squares method, using MULTI [19] with the Damping–Gauss–Newton algorithm according to the following equations;

$$C_b = B_{max} \times C_f/(K_d + C_f),$$

$$\% \text{ of control} = (K_d + C_f) \times 100/(K_d \times (1 + I/K_i) + C_f)$$

where C_b is the [^{3}H]mepyramine-specific binding concentration, C_f is the unbound concentration of [^{3}H]mepyramine, and I is the concentration of unlabeled H1 antagonists.

The k_{on} and k_{off} of the H1 antagonists for human H1 receptor were also determined, as previously described [18]. Dissociation of [^{3}H]mepyramine was induced by adding 10 μM cetirizine after incubating membranes with 3 nM [^{3}H]mepyramine for 30 min to determine k_{off}. k_{on} was then obtained by fitting to account for the unbound and bound ligand drug concentration–time profiles. To determine the k_{on} and k_{off} of unlabeled H1 antagonists, the association kinetics of [^{3}H]mepyramine were measured in the presence of a concentration of unlabeled H1 antagonists that inhibited [^{3}H]mepyramine binding at equilibrium by approximately 80%. Data were analyzed according to the model proposed by Motulsky and Mahan [20] using Phoenix™ WinNonlin® version 6.1 (Pharsight Corp., Mountain View, CA).